EXHIBIT 1

KAROLINSKA INSTITUTET
a medical university

Department of: CLINICAL [illegible]

Laboratory Journal No:

Name: [redacted]

Group:

Date: from to

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Study

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Project no.

Study no. 27

Assembly of a synthetic gene coding for Fel d 1 Chain 1 using Taq

PCR

Oligos 132, 133, 134, 135 10 µM

1 µl of each oligo 132-135
1 µl dNTP 10 mM
0.5 µl Taq
1 µl 10x Taq-buffer
35 µl H_2O

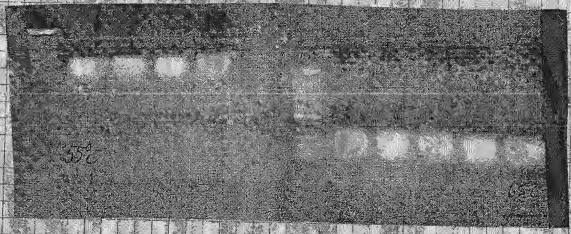
10 µl

→ PCR Eppendorf program HANS1

94°C 1 min
annealing 53-65°C 15 min (gradient)
elongation 68°C 20 min

30 cycles and ang 10 min elongation
+4°C

Result: A strong band around 300 (exp. ~280)



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Laboratory Journal 248910

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Study: Assembly of chain 2 Fel d1

Project no.

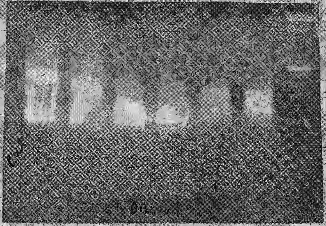
Study no.

primers 127-131 + 138

Using 3 different DNA polymerases: Taq, pfu, Ampli Taq

Methodes: "assembly chain 1"

expected band at 294



-300

to 248913 lane 1

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Study: Expression and purification of Fel d1 chain 1 and chain 2

Project no.

Study no.

Fel d1 chain 1 (clone 42) and Fel d1 chain 2 clone 29 was ligated into pET 20b and electroporated into BL-21 DE3 plysS after having been cut from pBT-Bluescript containing the correct sequence (see binder HG1, Fel d1) Sequencing of pET 42/pET 29 was done according to standard protocol (ABI) and the result can be seen on the opposite side.

Both Fel d1 chain 1 (Fd1:1) and Fel d1 chain 2 (Fd1:2) was expressed according to standard protocol and purified on a thiol (chelate) column loaded with $NiSO_4$.

Chain 1 was soluble, after ultrasonication chain 1 was found in 20 mM Tris HCl pH fraction, while chain 2 was found in the inclusion bodies after "washing" with 2M Urea buffer + 20mM Tris HCl pH 8.0. The inclusion bodies were solubilized in 6M Guanidine, transferred to 6 M Urea buffer (20 mM Tris-HCl pH 8.0 + 0.5M NaCl) via 6 ml HiTrap. Purification was done on FPLC

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Luftsensibilisering Blad: Allergi II

Personnummer	ldnr	g.1	e1 prick	
340217-3235	29	0.47		Atopiker
370413-3218	59	11.4	3	Atopiker
380620-1236	68	9.2	3	
410317-3210	95	1.24		
420509-3215	102	2.21		
421013-3254	110	0.43		
430306-3277	118	1.3		
441118-3272	135	1.03	2	
471031-3273	172	1.07		
490710-3214	199	0.92		
500226-3217	203		2	
500515-3233	204	0.73		
510414-3231	215		2	
511020-3212	220	0.64	3	
531016-3232	243	0.37		
531106-7838	245	0.89	2	
540208-3214	253		2	
540823-9332	261	67.7	3	
550521-3213	277	28.5	2	Atopiker
560701-3218	283	0.68		
570415-3211	285	0.6[illegible]		
[illegible]	312	0.82	2	
[illegible]	343		3	
[illegible]	352	2.65		
621119-3252	375		2	
650127-3212	380		2	
630703-3230	385	1.55		
640724-3234	398	0.47		
650523-3210	403		2	
650626-3212	406	0.76		
660703-3211	416	3.06	2	
671004-3255	422	25.5	3	Atopiker
680220-3213	426		2	
680520-3210	428	0.50		
690108-3235	434	21.45	3	Atopiker
730812-3251	452	5.33		
740306-0044	454	8.33	2	Atopiker
740313-3243	455	0.36		
750109-3210	456	17.1	3	

Σ 31 st

C:\program\genesis\protocol\abs3\07NOV00W.004

```
LABSYSTEMS GENESIS V2.xx  Results
Raw data filename       : C:\program\genesis\protocol\abs3\(
Processed by Protocol   : C:\program\genesis\protocol\abs3.prt
Plate layout file       : C:\program\genesis\protocol\abs3.plt
Reading type            : Dual Wavelength
Instrument version      : Multiskan RC V.3.0
Filter 1                : 405nm
Filter 2                : 620nm
Dag time                : 00.00:10
Interval between rdgs   : 00:00:10
Mix                     : YES
Mix RPM                 : High
Mix ON period           : 00.00:05
Mix OFF period          : 00:00:05
Comments:
Platta 1 45 min
```

Raw data values (calculated):

	1	2	3	4	5	6	7	8	9	10	11	12
A	[illegible]	0.172	0.317	0.058	2.039	0.364	0.475	0.128	0.140	0.053	0.292	0.197
B	[illegible]	[illegible]	0.394	2.226	1.801	[illegible]	[illegible]	0.156	0.152	0.098	0.320	0.192
C	[illegible]	[illegible]	0.347	0.477	1.425	0.066	[illegible]	0.148	0.138	[illegible]	[illegible]	[illegible]
D	[illegible]	0.008	0.008	0.007	0.008	[illegible]	0.008	0.008	0.008	[illegible]	[illegible]	0.008
E	0.006	0.008	0.006	0.008	0.008	0.008	0.007	0.008	0.008	[illegible]	0.008	0.008
F	0.006	0.008	0.008	0.009	0.009	0.008	0.008	0.009	0.007	0.004	0.008	0.008
G	0.007	0.007	0.007	0.007	0.008	0.009	0.008	0.007	0.009	0.008	0.007	0.008
H	0.008	0.007	0.006	0.006	0.008	0.008	0.007	0.008	0.010	0.010	0.008	0.008

(29) kontroll (59) (277) (422) (434) (29) (454)

kU/L 0,47 11,4 28,5 25,5 21,45 8,3

Atopi

```
Comments:
Platta 1 45 min
```

Raw data values (calculated):

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.049	0.057	0.169	0.112	1.395	0.817	0.537	0.253	0.197	0.088	[illegible]	0.098
B	0.044	0.053	0.316	0.096	1.403	0.689	0.643	0.248	0.135	0.063	0.146	0.117
C	0.056	0.051	0.178	0.056	1.137	0.445	2.764	0.958	0.114	0.020	0.158	0.102
D	0.051	0.386	0.182	0.105	1.162	0.776	[illegible]	0.219	0.179	0.055	0.149	0.192
E	0.054	0.133	0.068	0.059	0.851	0.411	2.409	1.092	0.114	0.028	0.148	0.094
F	0.051	0.042	0.066	0.234	0.837	0.532	2.477	1.118	0.211	-0.043	0.179	0.088
G	0.063	0.050	(0.387)	0.108	1.435	0.523	0.497	0.258	0.182	0.086	0.153	0.120
H	0.053	0.119	0.193	(0.250)	1.140	1.154	1.916	0.046	0.107	0.048	0.297	0.089

K1 (rows A–D), K2 (rows E–H)

24 59 277 422 434 457

Coatning: A = 10 µg/ml
B = 5 µg/ml
C = 2,5 µg/ml
D = 1,25 µg/ml

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Study

Project no.

Study no.

Test of Fd 1 chain 1, clone 42 and
Fd 1 chain 2 clone 29 with 6 cat neutralized
biopsies from Algot study

6 persons from the ALGOT study, no 29, 59, 277, 422, 434 and 454 was titrated 3 times and 10 times resp. in PBS, pH 7.4. A μ-titer plate was coated with:

Plate 1

A	20 μg/ml chain 1	→ (All horizontal wells)
B	5 μg/ml	
C	2.5 μg/ml	
D	1.25 μg/ml	
E	10 μg/ml chain 2	→
F	5	
G	2.5	
H	1.25	

Plate 2

A	5+5 μg/ml	chains 1+2 (a 1:1 mix of resp chains
B	2.5+2.5	chain 1+2 (1% 5 μg/ml each)
C	1.25+1.25	

On the vertical rows the patients were added

1	2	3	4	5	6	7	8	9	10	11	12
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Patient 29		59		277		422		434		454	
3x	10x	3x	10x								

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Study: cont.

Project no.

Study no.

ELISA conditions

Coating of Fd 1:1 and Fd 1:2 (100 µl/well) over the weekend in +4°C. ~~Serum~~ 4 times wash with "Tvättlösning"
Patients serum was added, 100 µl/well and inkubated at +4°C o.n. Wash 4 times (Wallac ELISA-washer) with "Tvättlösning" and 100 µl Rabbit α human ~~anti~~ IgE dil 1000 times in "Vår buffert)
Inkubation 2 hours in RT on shaker, Wash 4X "tvättlösning" and ad 100 µl/well of Goat anti-rabbit-ALP konjugated (DAKO) for 1h
Wash 4 times and ad substrate 3 tablets/15 ml of Vår buffert.
The result was read in ELISA reader after 45 min at 405 ~~nm~~ nm

Result: 2.5 µg/ml seems to be an adequat coating concentration for both chain 1 and chain 2. Mixing of the two chains can be done with coating concentrations 2.5 + 2.5 µg/ml

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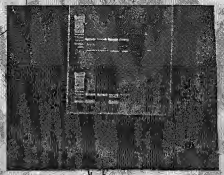
1a 1b 2a 2b

00114



1 2a 3b 4a 4b

(1a) 1µl template clone U.42 (1:1000)
2µl primer 176
2µl ___ 174
2µl 10× buffy
1µl pfu
2µl dNTP (10mM)
10µl H_2O
20µl

(1b) Som ovan men primer Taq polymeras

(2a) 1µl templ. (1:1000) klon 46
1µl templ ___ klon 29
2µl primers 176, 174, 183
i övrigt som (1a)

(2b) Som (2a) men Taq

(3a) 1µl templat klodja 2 (1:1000)
2µl 174
2µl 175
i övrigt som 1a

(3b) Som (3a) men Taq

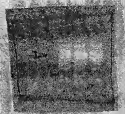
(4a) 1µl templ. 1
1µl templ. 2
2µl primer 160
i övrigt som (3a)

(4b) Som (4a) men Taq

Putting clean 1 and 2 (1+2) together (00124)

template 1a 6µl
template clone 29
1:10, 1:100, 1:1000

1µl	
0,5µl	primer 176
2µl	___ 183
2µl	dNTP
3µl	10× buffy
1µl	pfu
14,5µl	H_2O
30µl	



1µl 1µl 1µl
1:10 1:100 1:1000

AmpliTaq Gold ™
250 Units, 5U/µl
Store at -20 °C

A03912

Result: One band of ca 500 bp as we should be clean 1+2

The bands are cut out and purified on Qiagen

ligated with perfectly blunt cloning kit. 10 colonies are picked for miniprep and possibly sequencing

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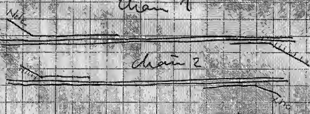
Study: Linking of (chain 1 and chain 2 (seamless)) with PCR Feld1:1 and Fel d1:2

Project no.:

Study no.:

Aim. The aim of this experiment is to join the two chains of Fel d1 into one construct by PCR

Study outline. The two sequenced chain of the major ~~chains~~ allergen of cat Fel d1 (chain 1, clone 42) and chain 2, clone 29) is joined with PCR i two steps as outlined below. In

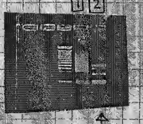


template 42 (plasmid Fd1:1 in pT7-Blue)

templ 29 (pl. Fel1:2 in pT7-B)

Result (see opposite side)

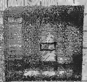
Good bands of expected size was seen for both chain 1 and chain 2. But (2a) + (2b) as well as (4a) and (4b) did not work. I will confirme by adding (1a) to ~~vector~~ template 29 and do PCR. (chain 1+2)



~~PCR:s 1 (1+2)~~
~~1µl templ. ch1 (1:1000)~~
~~2µl 176~~
~~2µl 174~~
→ did not work

PCR primers 180, 181

PCR 2 (2+1)
1µl template chain 1 (1:1000)
1µl chain 2 (1:1000)
2µl 181
2µl 175
2µl 180
1µl Pfu
3µl 10x buff
dNTP
H2O → Σ 30µl



← cut band

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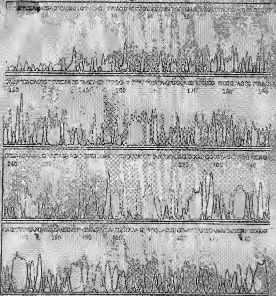
PRISM ABI 100 Version 3.2 Lane 25



Td1 (2+1) pT7 d1R 001205 (t7 term.)

2+1 z ⟶

ABI PRISM

Model 377 Version 3.4 ABI100 Version 3.2

27•Fd1 2+1 pT7 d1R

Fd 1 2+1 pT7

Lane 27

Signal G:112 A:183 C:111 T:126

DT (dR Set Any-Primer)

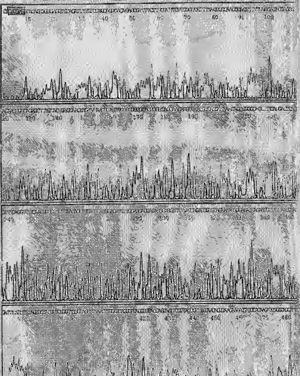
Seq dRhod E 182 extra

Points 1335 to 8260 Pk 1 Loc: 1335

mån 4 dec 2

mån 4 dec 2

Spacing



Laboratory Journal

248931

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Project no.

Study no.

Study: sequencing of 4 clones of each 1+2 and 2+1.

1+2 — clone no ↓ — seq no ↓ — 2+1

Clone	1	= (1)	clone	1	(5)	OK seq
	2	= (2)		6	(6)	
	5	= (3) OK seq		7	(7)	OK seq (poor seq)
	9	= (4)		9	(8)	

red = prom.
black = term.

4.8 µl vektor
1.2 µl primers
4 µl mix BD eller rhodamin
10 µl

+ 50 µl oil and

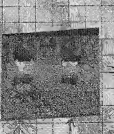
PCR på Perkin Elmer
96°C 30''
50°C 15''
60°C 4'
} 25 cycles

Unfortunately there was a scheduled power failure and the PCR-run was interupted. Assume 7 more cycles which is done.

Samples are loaded on lanes 20 – 33 on ABI seq and named Fd1 1+2 pT7 clone 4F
— " — 4R
— " — 5F
— " — 5R
etc.

Cutting of clone 5 (1+2)
and clone 1 (2+1)
with Not1 and Xho1
for ligation into pET 20b

20 µl plasmid mini prep (pT7 Blue)
2.4 µl 10x buffer
0.3 µl BSA
0.7 µl Not1 Incubate 37°C shake
0.7 µl Xho1 for 2h.



→ Qiagen purif.

clone 1(2+1) clone 5 (1+2)

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Study: Ligation of Fd 1, klone 1 (2+1) and clone 5 (1+2) into pET 20b+ and electroporation into BL21 plys

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Project no.

Study no.

The fragments from Fd 1 (2+1) klone 1 and clone 5 (1+2) were Qiagen purified from 1% agarose gel (248931)

A cleaved (Nde/Xho) pET 20b+ vector was used to ligate the fragments

Conditions:

~~vector~~ 12 µl fragments
1.0 µl vector
1.5 µl 10 mM ATP
1.8 µl 10x ligase buffer
1.7 µl T4 ligase
18 µl

Ligate +16°C o.n.

The ligate mix was electroporated into 50 µl BL21-plys electrocompetent cells. 1 µl ligate mix was added to thawed cells (on ice). Electroporation according to standard protocol. Growth on SOC medium for 60' 37°C shake (300 rpm) and plated on Amp/CAm plates. One colony ~~on~~ from each clone was picked and grown on LB Amp Cam medium, miniprepped (Qiagen) and 25 µl of the (50 µl) prep was cut with Nde and Xho. Result

clones

~ 800 bp

Both clones contain the insert !!

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Project no.

Study no.

Study: Purification of Fd 1 (1+2) clone 5 and Fd1 (2+1) clone 1 over Ni⁺ chelate Hi-Trap

(in pET 20b in BL-21 pLysS)
1 Liter of Fd1 (1+2) and (2+1) resp. LB-medium (CAM, Amp) was grown to OD 0.6 (600 nm) and induced with 0.4 mM IPTG. (see 248932)

Purification according to protocol. Both protein were expressed as inclusion bodies and purified accordingly. Purification on FPLC. as follow. After adsorption onto Ni-column in 6M Gua and wash also with 6 M Gua the column is stuck to FPLC.

Program:

0 conc %B 0 —— A = 6 M Urea

0 ml/min 5.0 ml/min B = 20 mM Imidazol

0 0.25 ml/frac

0 port set 6.1 C = 500 mM Imidazol

20 conc %B 0

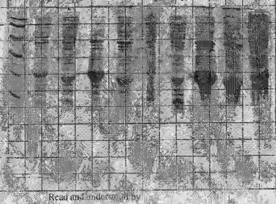
80 conc %B 100

100 conc %B 100

125 conc %B 0

125 port set 6.0

LMW



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